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*Editorials*

# D, weak D ($D^u$), and partial D: the molecular story unfolds

Molecular cloning of Rh cDNA and subsequent studies of both genomic DNA and cDNA have revealed much about the structures of the *Rh* genes and the proteins they encode. In 1991, Colin et al.[1] reported that individuals with D+ red cells have two *Rh* genes, *RHD* and *RHCE*, while most of those with D– red cells have only one gene, *RHCE*. *RHCE* is the name of the gene whose alleles include *RHCe*, *RHcE*, *RHce*, and *RHCE*; most individuals with D– red cells are homozygous for *RHce*. The *RHD* and *RHCE* genes are closely aligned on chromosome one, and each comprises 10 exons. In D– persons, a lack of D antigen almost always represents deletion of *RHD*, although rare D– individuals have one or more detectable but nonfunctional *RHD* genes.

From this information, a better understanding of the weak D ($D^u$) and partial D phenotypes has emerged. It has long been known that D may be weakly expressed on red cells; the phenotype involved was named $D^u$ by Stratton[2] in 1946. Most individuals of this phenotype are unable to make alloimmune anti-D, which suggests that their red cells carry all epitopes of D. In contrast, a small subset of persons with D+ red cells are able to make anti-D that reacts with all normal D+ red cells but not with their own or with those of other individuals of the same unusual D+ phenotype.[3] Various terms have been used to denote this situation: the most descriptive is that introduced by Tippett,[4] who described these persons as having partial D on their red cells. In partial D phenotypes, one or more epitopes of D are missing, and such persons can produce alloimmune anti-D against the epitope(s) of D that their red cells lack. However, the partial D phenotypes may, but do not necessarily, involve recognizably weakened expression of D.

The $D^u$ phenotype—or, as it is better described,[5] the weak D phenotype—involves a quantitative variation of D. Studies with both polyclonal and monoclonal anti-D have been used to determine the number of D antigen sites on red cells. Normal D+ red cells of the $R_1r$ and $R_2R_2$ phenotypes bear about 10,000 and 30,000 D antigen sites per cell, respectively.[6,7] In contrast, on weak D red cells, D antigen sites number from 300 to 9000 per cell.[8-10]

The partial D phenotype always involves a qualitative difference in the D antigen and sometimes leads to a quantitative difference (partial weak D or partial $D^u$ phenotype) as well. In tests using potent anti-D typing reagents made from pools of human polyclonal anti-D, most partial D red cells type as unremarkable D+. When polyclonal anti-D was used to examine four samples of partial D category VI red cells, the number of D sites per cell was found to vary from 1700 to 3200.[9] However, three samples of partial D category V red cells had 13,000 to 17,000 D antigen sites per cell (i.e., more D sites than are found on some normal D+ cells of the $R_1r$ phenotype).[9] In tests using a monoclonal anti-D and five samples of partial D category VI red cells, the number of D sites varied from 3,000 to 11,000.[11]

**See related article, page 104**

Relatively little is known about the mechanism(s) that causes downregulation of D expression in the weak D phenotype when all epitopes of D are expressed. Beckers et al.[12] recently reported studies on six samples with weak D, in which it had been shown that the number of D antigen sites per red cell was between 500 and 1000; four of the samples typed CcDe and two typed cDEe. All six individuals had *RHD* that was grossly normal when studied by Southern blot analysis and by polymerase chain reaction amplification of selected regions of *RHD*. Analysis of Rh transcripts demonstrated normal, full-length *RHD* cDNA. Thus, no obvious mechanism was apparent to explain the low number of copies of D on the weak D red cells of these individuals with apparently normal *RHD* genes. Clearly, additional studies will be required to determine whether an inefficient transcriptional process, abnormality in posttranscriptional regulation, an inefficient translation process, or some other mechanism is at play. Eventually, the explanation for the weak D phenotype in the presence of a normal *RHD* gene may also throw light on the well-established finding that expression of D is affected by the other gene in the Rh

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haplotype. A single example is that, in the genotype $R^1R^1$, red cells carry between 15,000 and 20,000 D sites per red cell, while, in the $R^2R^2$ genotype, the number varies from 16,000 to 34,000.[6,7] Clearly *RHD* expression is regulated differently in the company of *RHCe* or *RHcE*.

Far more is known about the molecular genetics of partial D. At the time of meiosis, misalignment of *RHD* and *RHCE* can result in gene conversion in which one or more exons of one gene replace the equivalent exon(s) of the other. Such gene conversion can result in formation of a gene that encodes a polypeptide that lacks some of the epitopes encoded by the unconverted gene, that is, the partial D phenotype. However, partial D phenotypes can also arise via gene deletion and mutation. Mouro et al.[13] studied the *RHD* genes in 10 individuals with partial D category VI red cells. In 8 of the 10, all of whom had the $R^1$ haplotype, exons 4, 5, and 6 of *RHD* were replaced by exons 4, 5, and 6 of *RHCE*. In the other 2 persons, both of whom had the $R^2$ haplotype, exons 4, 5, and 6 of *RHD* were deleted. Rouillac et al.,[14] Colin et al.,[15] and Cartron[16] reported additional data. In category IVa, exon 3 and perhaps exon 7 of *RHD* are replaced by exon 3 and perhaps exon 7 of *RHCE*. In category IVb, exons 7, 8, and 9 of *RHD* are replaced by exons 7, 8, and 9 of *RHCE*. In category Va, exon 5 of *RHD* is replaced by exon 5 of *RHCE*.

Elucidation of the genetic event giving rise to the partial D category IIIb phenotype also provided information about G antigen expression. All red cells that are C+ are also G+, and almost all D+ red cells are also G+. In contrast, almost all D–,C– cells are G–. However, the very rare phenotypes D+,G– and D–,G+ exist. Partial D category IIIb red cells are D+,G–.[3,4] Rouillac et al.[17] showed that, in persons with category IIIb red cells, exon 2 of *RHD* is replaced by exon 2 of *RHCE*. Exons 2 of *RHD* and those alleles of *RHCE* that encode C but not c are identical, while the alleles of *RHCE* that encode c but not C (i.e., *RHce* and *RHcE*) differ from exon 2 of *RHD*. The G antigen is encoded by the *RHD*, *RHCe*, and *RHCE* genes but not by *RHce* or *RHcE*. The finding that category IIIb cells are D+,G– thus correlates with the finding that exon 2 of *RHD* has been replaced with exon 2 of *RHce* or *RHcE*. Thus, in category IIIb red cells, the lack of G as well as the lack of certain epitopes of D can be explained by a single genetic event.

Beckers et al.[18] recently added data regarding the molecular genetics of partial D. In category IIIc, exon 3 of *RHD* is replaced by exon 3 of *RHCE*. However, partial D category IIIc red cells carry all epitopes of D thus far recognized[19,20]; 30 such epitopes may exist.[21] That category IIIc red cells truly carry partial D is evidenced by the ability of persons of that phenotype to make alloimmune anti-D.[3,4] Apparently, the epitope(s) of D encoded by exon 3 of *RHD* have not yet been characterized. In the partial D phenotype, DBT, exons 5, 6, and 7 (and possibly 8) of *RHD* are replaced by the equivalent exons of *RHCE*.

The $R^{oHar}$ haplotype is discussed below. Just as some partial D category VI samples result from gene deletion rather than gene conversion,[13] category VII differs from other partial D categories elucidated thus far. The RhD polypeptide in category VII differs from that of normal D+ cells in that a thymine to cytosine nucleotide substitution at position 329 results in an amino acid change from leucine to proline at residue 110 of the D protein.[22] Thus, three different genetic mechanisms have already been shown to result in partial D phenotypes.

Rouillac et al.[23] suggested that it may prove possible, at least in some instances, to correlate the absence of D epitopes at the phenotypic level with the replacement of *RHD* exons at the molecular genetic level. Thus, it may be possible to suggest which exons encode which D epitopes. Support for this concept has been provided by Beckers et al.[18]

Elucidation of the genetic mechanisms that underlie partial D phenotypes might also explain how certain low-frequency Rh antigens arise. When an exon from one *Rh* gene replaces the equivalent exon in another *Rh* gene, or when exon deletion, missplicing, or point mutation occurs, the resultant polypeptide encoded will have an amino acid sequence different from that encoded by the wild-type *RHD* or *RHCE* genes. If the new sequence of amino acids is immunogenic, it may stimulate production of an antibody that recognizes this rare protein structure. It seems entirely probable that the antigens $Go^a$, $D^w$, Rh32, Rh33, Rh45, Rh50 (FPTT), and BARC, all of which can be associated with various categories of partial D, represent such events. Similarly, the Rh40 (Tar) antigen found on partial D category VII red cells would seem to be formed by the leucine to proline change mentioned above.

One altered *RHD* gene that fails to encode any epitopes of D has been found. The gene $r'^s$ can be variously written as $Cce^s$ or $ce^sC^G$. Because it produces no D, it had long been assumed to be a variant form of $r'$ or $r$. However, Blunt et al.[24] showed that $r'^s$ is derived from *RHD*. Their work showed that the $r'^s$ gene contains at least exons 1, 2, 8, 9, and 10 of *RHD*; because the other chromosome carried a normal *RHce* gene, it was not possible to determine whether exons 3 to 7 had been deleted or replaced with exons 3 to 7 of *RHce*. As no genetic material from *RHCE* likely to encode C was found, the authors suggested that the weak and somewhat atypical C encoded by $r'^s$ was a product of a remaining *RHD* exon.

The $R^{oHar}$ gene has long been an enigma for blood group serologists. When first described,[25] it was reported to encode normal c; reduced amounts of D, e, f, and $Hr_o$; a new low-incidence antigen Rh33; and no $hr^s$, G, or Hr. The D antigen encoded by $R^{oHar}$ was difficult to detect with most polyclonal anti-D made by D– persons, and many early examples of red cells from persons genetically $R^{oHar}/r$ were initially thought to be D–.[26,27] Surprisingly, occasional polyclonal anti-D reacted well with the D

made by $R^{oHar}$. More recently, some monoclonal anti-D reacted as if the red cells of persons with $R^{oHar}$ carry normal D. Because no individual who is genetically $R^{oHar}/r$ had ever been shown to make anti-D, there was no information as to whether the D antigen made by the gene simply was quantitatively different from normal D or whether it had qualitative differences as well.

An article from Beckers et al.[28] in this issue of **TRANSFUSION** has now firmly established that D encoded by $R^{oHar}$ is partial D. First, the red cells of three unrelated individuals in whom $R^{oHar}$ was partnered by a chromosome that lacked *RHD* were shown to carry the D epitopes epD5 and epD6/7 but to lack epD1, 2, 3, 4, 8, and 9. Second, one of the three individuals had made alloimmune anti-D that did not react with the D+ red cells of the other individuals with $R^{oHar}$. Thus, Rh33 is shown to be a product of another gene that makes partial D; it had previously been seen to be made by $D^{IV}(C)-$.[29] In addition to the results presented in the current article,[28] Beckers et al.[18] have showed that the $R^{oHar}$ gene is actually an *RHce* gene in which exon 5 of *RHce* is replaced by exon 5 of *RHD*. As reviewed above, previously characterized genes that encode partial D have often been *RHD* in origin, with partial substitution with exons from *RHCE*. The reverse seems to have occurred in $R^{oHar}$. An *RHce* gene seems to have been partially converted to an *RHD* gene by replacement of one of its exons with an equivalent exon from *RHD*. Because the converted gene carries exon 3 of *RHce*, nonproduction of G by $R^{oHar}$ is explained.

Since the first example of anti-D was described but not named 57 years ago,[30] the Rh system has been particularly stubborn in its reluctance to divulge the secrets of its genetic basis. Thus, it is astonishing that so much has been learned in the 14 short years since the first successful isolations of the Rh polypeptides were reported.[31,32] As this brief commentary shows, the clinically important D antigen is now largely understood in terms of the molecular genetics of D, weak D (formerly $D^u$), and partial D. With blood group serologists working in concert with molecular biologists, the rest of the story will surely follow in short order.

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